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*January 07, 2005*

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APPLICATION NUMBER: 60/527,330

FILING DATE: *December 05, 2003*

RELATED PCT APPLICATION NUMBER: *PCT/US04/40660*



Certified By

Jon W Dudas

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# **PROVISIONAL APPLICATION FOR PATENT COVER SHEET**

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53 (c).

INVENTOR(S)					
Given Name (first and middle [if any])		Family Name or Surname		Residence (City and either State or Foreign Country)	
Julia Y. Eggehard		Ljubimova Holler		Los Angeles, California Regensburg, Germany	
<input type="checkbox"/> Additional inventors are being named on the _____ separately numbered sheets attached hereto					
TITLE OF THE INVENTION (280 characters max)					
DELIVERY OF ANTISENSE OLIGONUCLEOTIDES AND TRANFERRIN RECEPTOR ANTIBODY IN VITRO AND ON VIVO USING A NEW MULTIFUNCTIONAL DRUG DELIVERY SYSTEM BASED ON POLYMALIC ACID					
CORRESPONDENCE ADDRESS					
Direct all correspondence to:					
<input checked="" type="checkbox"/> Customer Number <b>000028983</b>					
OR Type Customer Number here					
<input type="checkbox"/> Firm or Individual Name		REED SMITH CROSBY HEAFEY			
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		Telephone	310 734-5200	Fax	310 743-5299
ENCLOSED APPLICATION PARTS (check all that apply)					
<input checked="" type="checkbox"/> Specification Number of Pages		2		<input checked="" type="checkbox"/> Small Entity Statement	
<input type="checkbox"/> Drawing(s) Number of Sheets		0		<input checked="" type="checkbox"/> Other (specify) return postcard Express Mail Certificate	
METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT (check one)					
<input type="checkbox"/> A check or money order is enclosed to cover the filing fees					
<input checked="" type="checkbox"/> The Commissioner is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number: 50-2567					
FILING FEE AMOUNT (\$) \$80					
The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.					
<input checked="" type="checkbox"/> No.					
<input type="checkbox"/> Yes, the name of the U.S. agency and the Government contract number are: _____					

Respectfully submitted,

SIGNATURE

*Stefan J. Kirchanski*  
Stefan J. Kirchanski

Date 12/5/03

TYPED or PRINTED NAME

310 734-5403

REGISTRATION NO.  
(if appropriate)

36,568

Docket Number:

356830.00500

TELEPHONE

## **USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT**

This collection of information is required by 37 CFR 1.51. The information is used by the public to file (and by the PTO to process) a provisional application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 8 hours to complete, including gathering, preparing, and submitting the complete provisional application to the PTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, Washington, D.C., 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Box Provisional Application, Assistant Commissioner for Patents, Washington, D.C., 20231.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

Julia Y. Ljubimova, et al.

Serial No: Not Assigned

Filed: December 5, 2003

For: DELIVERY OF ANTISENSE  
OLIGONUCLEOTIDES AND TRANFERRIN  
RECEPTOR ANTIBODY IN VITRO AND ON  
VIVO USING A NEW MULTIFUNCTIONAL DRUG  
DELIVERY SYSTEM BASED ON POLYMALIC  
ACID

Art Unit: Not Assigned

Examiner: Not Assigned

CERTIFICATE OF MAILING VIA U.S. EXPRESS MAIL  
"Express Mail" Mailing Label No. EV 228462431 US  
Date of Deposit: December 5, 2003

Mail Stop PROVISIONAL PATENT APPLICATION  
Commissioner for Patents  
P.O. Box 1450  
Alexandria VA 22313-1450

Dear Sir:

I hereby certify that

- ☒ two copies of PROVISIONAL PATENT APPLICATION
- ☐ check in amount of \$\_\_ as filing fee
- ☒ patent application ( 2 page(s) of specification; 0 claim(s); 0 page(s) of abstract
- ☐ \_\_ sheet(s) of ☐ formal ☐ informal drawings
- ☐ executed Declaration and Power of Attorney (☐ copy from parent)
- ☐ assignment of the invention to \_\_ (☐ copy from parent)
- ☐ certified copy of \_\_ patent application No. \_\_ which was filed \_\_ from which priority is claimed in the subject case pursuant to 35 U.S.C. § 119
- ☐ Preliminary Amendment
- ☒ Assertion of Small Entity Status under 37 CFR 1.27
- ☐ Request And Certification Under 35 U.S.C. 122(b)(2)(B)(i) for non-publication
- ☒ return postcard

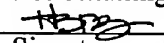
are being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service with sufficient postage under 37 C.F.R. § 1.10 on the date indicated above and are addressed to:

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Washington, D.C. 20231.

Date: December 5, 2003

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Signature

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December 5, 2003

**VIA EXPRESS MAIL, LABEL NO. EV 228462431 US**

Mail Stop PROVISIONAL PATENT APPLICATION  
Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313

Re:    ASSERTION OF SMALL ENTITY STATUS  
      New U.S. Provisional Patent Application  
      Inventor(s)           : Julia Y. Ljubimova, et al.  
      DELIVERY OF ANTISENSE OLIGONUCLEOTIDES AND TRANSFERRIN RECEPTOR  
      ANTIBODY IN VITRO AND ON VIVO USING A NEW MULTIFUNCTIONAL DRUG  
      DELIVERY SYSTEM BASED ON POLYMALIC ACID  
      Filed                 : December 5, 2003  
      Attorney Docket No. : 356830.00500

Dear Sir or Madam:

The undersigned attorney hereby asserts that the Patent Application entitled "DELIVERY OF ANTISENSE OLIGONUCLEOTIDES AND TRANSFERRIN RECEPTOR ANTIBODY IN VITRO AND ON VIVO USING A NEW MULTIFUNCTIONAL DRUG DELIVERY SYSTEM BASED ON POLYMALIC ACID" by Julia Y. Ljubimova, et al. filed on December 5, 2003 is entitled to Small Entity Status. The required inquiry to establish Small Entity (§ 1.27(a)) status has been made.

Very truly yours,



Stefan J. Kirchanski

SJK/hdb

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r e e d s m i t h . c o m

"Reed Smith" and "Reed Smith Crosby Heafey LLP" refer to Reed Smith LLP and related entities.

**APPLICATION FOR  
UNITED STATES PROVISIONAL PATENT**

**INVENTED BY**

Julia Y. Ljubimova

&

Eggehard Holler

Assigned to:

Arrogene, Inc.

**for**

**Delivery of antisense oligonucleotides and transferrin receptor antibody in vitro and  
in vivo using a new multifunctional drug delivery system based on polymalic acid**

prepared by:

**Crosby, Heafey, Roach & May  
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**Attorney Docket No. 356830.00500**

## Delivery of antisense oligonucleotides and transferrin receptor antibody in vitro and in vivo using a new multifunctional drug delivery system based on polymalic acid

### **Introduction:**

Specific targeted drug delivery is crucial for treating tumors and reducing side effects to normal cells. Simultaneous inhibition of a plurality of molecular targets and at different steps of the protein synthesis process can be more effective in preventing tumor growth and progression than inhibition of a single protein synthesis step.

### **Methods:**

We have designed and constructed a multifunctional drug delivery system consisting of a molecular construct comprising molecular modules attached to the pending carboxyl groups of a polymalic acid backbone. The polymalic acid polymer is a natural product of *Physarum polycephalum*. The modules are (1) Morpholino antisense oligonucleotides attached to the scaffold by a disulfide linkage, which is cleaved in the cytoplasm to release the free drug, (2) an antibody against transferrin receptor for cancer cell targeting and receptor-mediated endocytosis, (3) short chain PEG-coupled L-leucine and directly coupled L-valine, both by amide bonds, to provide pH-dependent lipophilicity to disrupt endosomal membranes, (4) long chain PEG for protection, (5) fluorescent reporters (fluorescein or Cy5) to detect the construct within tissues and cells. Drug penetration was detected immunohistochemically using U87-MG glioblastoma multiforme cell line *in vivo* and *in vitro*. Double staining was performed to visualize transferrin receptor antibodies in conjunction with the fluorescein reporter.

### **Results:**

The functional effect of module 1 (Morpholino antisense against laminin chains) was detected by immunostaining for laminin alpha 4 and beta 1 laminin chains. The antisense oligonucleotide should block the synthesis of these laminins. Immunostaining showed significant difference between drug-treated and untreated tumors. Laminin protein synthesis was blocked both *in vitro* and *in vivo*. The functional effect of module 2 (antibody against transferrin receptor) was detected in cell cultures. The labeled polymalic acid and transferrin receptor antibody were visible in the cytoplasm equally at different time points (1 and 3 hours) after incubation. *In vivo*, similar results were achieved on frozen sections of rat brain tumor cells, which were stained more intensely than non-tumor brain cells. This indicates that the targeting effect of the transferrin receptor antibody was

discernible. The drug itself was not toxic in three different concentrations *in vitro* and *in vivo*.

**Conclusion:**

A multifunctional drug delivery construct based on polymalic acid can be efficacious for transferring antisense oligonucleotides together with monoclonal antibodies into the tumor cell probably using the mechanism of receptor-mediated endocytosis and endosomal rupture. Antisense effect in blocking the laminin protein synthesis was confirmed both *in vitro* and *in vivo*.